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# **Plant-microbial synergism: An effective approach for the remediation of shale-gas fracturing flowback and produced water**

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## Abstract

Effective and affordable treatment of hydraulic fracturing flowback and produced water (FPW) is a major challenge for the sustainability of unconventional shale-gas exploration and development. We investigated the effectiveness of different combinations of activated sludge (AS), three microbial preparations, and ten plants (ryegrass, water dropwort, *Typha*, reed, Iris, Canna, water caltrop, rape, water spinach, and *Alternanthera philoxeroides*) on the treatment performance of FPW. Water quality parameters ( $\text{NH}_4\text{-N}$ ,  $\text{NO}_3\text{-N}$ ,  $\text{NO}_2\text{-N}$ ,  $\text{COD}_{\text{cr}}$ , and BOD) and the algal toxicity of the treated FPW were measured to assess the treatment efficiency. The results showed that AS had higher treatment efficiency than the prepared microorganisms, and water dropwort was the best plant candidate for boosting performance of AS treatment of FPW. The treated FPW showed improved water quality and microbial diversity. The Shannon-Wiener index increased from 4.76 to 7.98 with FPW treatment. The relative abundance of microbes with a greater resistance to high salt conditions, such as *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, et al, increased substantially in the treated FPW. The combination of water dropwort and AS showed the greatest improvement in water quality and subsequently the highest algal density and microbial diversity, which indicated good potential for this candidate in the treatment of FPW.

**Keywords:** shale gas hydraulic fracturing; flowback and produced water; phyto-micro combined treatment; microbial diversity

## 1. Introduction

The widespread exploration and rapid development of unconventional shale gas generates a number of environmental issues; in particular, a high demand for water during the fracturing process and chemicals used in the drilling fluids can lead to irreversible groundwater and surface water contamination and high potential risks for human health [1-3]. Hydraulic fracturing for shale gas consumes millions of litres of water (7000~21000 m<sup>3</sup>/well) and accordingly produces high volumes of effluents (8~70% of the injected water) with variable pollutant compositions [1, 4, 5]. Effluents from hydraulic fracturing can be distinguished as two types: flowback water from the fracturing stage and produced water from the gas production stage [1]. It is a common practice to re-use these effluents instead of fresh water in subsequent hydraulic fracturing operations to reduce the costs of water treatment and minimize the environmental impacts of these effluents. However, studies have identified 1117 chemicals in these effluents, and 49 of these are probable or possible human carcinogens [2]. High concentrations of total dissolved solids (TDS), total suspended solids (TSS), organics (chemical oxygen demand (COD) and biological oxygen demand (BOD<sub>5</sub>)), aromatic compounds, heavy metals (Li, Ni, Pb, Cu, Zn, Mo, and Rb), various ions (Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, Fe<sup>2+</sup>, Na<sup>+</sup>, and Ca<sup>2+</sup>) and other pollutants are mixed together at variable compositions, which results in varied salinity, hardness, viscosity and oil and organic contents of the flowback and produced water (FPW) [2, 6-10]. Due to the complex and ever-changing compositions of FPW, it is one of the most challenging industrial sewage to dispose of properly [1, 11]. Thus, it is necessary to find effective yet economical solutions for FPW treatment to enable sustainable development of this rapidly growing unconventional energy source.

The existing options for disposal and minimization of FPW environmental impacts include filtration, pH adjustment, sedimentation and degreasing/de-oiling which are frequently used physical pre-treatments to remove TSS (proppant and other solids from underground) and reduce the salinity of FPW [1, 12]. However, water quality improvement is still very limited and volatile pollutants may escape during the treatments [1, 12]. The additions of coagulants and flocculants are effective chemical precipitation methods that minimize hardness, TOC and iron concentrations in FPW by softening the water, but these methods are often expensive [13-16]. Moreover, the treatment efficiency of FPW is usually quite poor for most of the pollutants and therefore limits the potential for water re-use. More effective techniques are needed to meet the requirements for discharge. Biological treatments have been broadly applied in the treatment of various types of sewage, especially high-salinity wastewater, and have showed positive effects on the biodegradability of dissolved organic

matter, the removal of nutrients (nitrogen and phosphorus) and suspended matter, as well as the absorption of metals [1, 17-19]. A mixed liquor of activated sludge (AS) was tested and demonstrated for treatment of FPW. A case study showed that an AS mixed liquor was capable of treating guar gum, which was a principal ingredient of the flowback water, with a removal efficiency that was greater than 60% with high TDS [20]. Other studies have found that FPW can be used to cultivate marine microalgae which could be a potential nature-based remediation option [21, 22]. In an earlier study, the effective removal of nitrogen and phosphorus was observed due to the uptake of these nutrients by algae [21]. Moreover, synergistic partnerships were found between microbes and plants in the remediation of some environments contaminated with toxic compounds [23-25]. Microbes had high potential to degrade organic pollutants and reduce the toxicity of hazardous chemicals, which can be beneficial for the improvement of plant health and growth. Plants can provide oxygen for microbial aerobic degradation of organic pollutants in addition to nutrients and microbial habitats associated with their dense and extensive root systems, which contributed to better survival and performance of the microbial community [23-25]. This study was intended to investigate a cost-effective biological treatment method for FPW to meet the Chinese Wastewater Discharge Standard (GB8978-1996) based on the combined effects of aquatic plants and microorganisms. Different combinations of four types of microorganisms and ten types of plants were tested for FPW treatment efficiency.

## **2. Materials and methods**

### *2.1 Wastewater collection*

Three types of FPW samples including one flowback water and two produced waters were collected. Flowback water (FW) and produced water 1 (PW1) were sampled in the Jiaoshiba shale-gas region located in Chongqing Province of China and produced water 2 (PW2) was collected from the Changning shale-gas region located in Sichuan Province of China. All the sampled FPW were kept in the dark at 4°C before use. All the collected samples were processed through centrifugal separation of hydrocarbon, water and solid before biological treatments. Aliquots from the supernatants were used in the treatment experiments.

### *2.2 Activated sludge and microorganisms*

Activated sludge was provided by the Cai-Dian domestic sewage treatment plant of Wuhan and transported to the laboratory for aeration overnight before application to FPW. The mixed liquor suspended solids (MLSS) concentration of the sampled activated sludge was obtained by standard MLSS analytical techniques [26]. The other three microbial preparations

(MP-N, MP-P, MP-R) were purchased from CLEAN-FLO, USA. The concentration of the microorganisms is shown in Table 1. The ten candidate plants (ryegrass, water dropwort, typha, reed, iris, canna, water caltrop, rape, water spinach, and *Alternanthera philoxeroides*) were provided by a local vegetable farm, and the roots of these plants were washed with deionized water. As shown in Table 2, different biomasses of the plants were used in this study to provide similar, approximate root volumes for each candidate plant. The root volumes were determined by the increase in water volume when the plants were placed in clean deionized water.

### 2.3 Analysis of chemical parameters of FPW

Water quality indices including total phosphorus (TP), phosphate, total nitrogen (TN), ammonia nitrogen ( $\text{NH}_4^+$ ), nitrite nitrogen ( $\text{NO}_2^-$ ), nitrate nitrogen ( $\text{NO}_3^-$ ) and chemical oxygen demand ( $\text{COD}_{\text{Cr}}$ ) of FPW were measured with a HACH DR2800 spectrophotometer following the procedures for the water quality analysis of water and wastewater described by Mukta et al [27]. Biochemical oxygen demand ( $\text{BOD}_5$ ) of FPW was measured with a HACH HQ30D dissolved oxygen meters, according to changes in the dissolved oxygen concentration over 5 days [28].

### 2.4 Aquatic ecotoxicity determination of FPW

Aquatic ecotoxicity is a comprehensive evaluation method developed by OECD [29], which is useful and acceptable for assessing the overall environmental effects of aquatic pollutants especially when multiple pollutants present simultaneously. *Scenedesmus obliquus*, a unicellular green algae with a rapid growth rate and high sensitivity to several contaminants [30, 31], was chosen for determining and comparing aquatic ecotoxicity of FPW before and after treatment based on the effects of FPW on algal growth and reproduction in 96 hours. The measurements were conducted according to the OECD Guideline 201, algal growth inhibition test [29]. First, four stock solutions of algal culture medium were prepared as follows: (1) a mixture of  $1.5 \text{ g}\cdot\text{L}^{-1}$  of  $\text{NH}_4\text{Cl}$ ,  $1.2 \text{ g}\cdot\text{L}^{-1}$  of  $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ ,  $1.8 \text{ g}\cdot\text{L}^{-1}$  of  $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ ,  $1.5 \text{ g}\cdot\text{L}^{-1}$  of  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$  and  $0.16 \text{ g}\cdot\text{L}^{-1}$  of  $\text{KH}_2\text{PO}_4$ ; (2) a mixture of  $80 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$  and  $100 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{Na}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$ ; (3) a mixture of  $185 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{H}_3\text{BO}_3$ ,  $415 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ ,  $3 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{ZnCl}_2$ ,  $1.5 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ ,  $0.01 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$  and  $7 \text{ mg}\cdot\text{L}^{-1}$  of  $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$ ; and (4) a solution of  $50 \text{ g}\cdot\text{L}^{-1}$  of  $\text{NaHCO}_3$ . All the reagents were analytical grade, and all the stock solutions were sterilized. Second, the algae were activated and pre-cultured for 2~3 days at  $20 \pm 2 \text{ }^\circ\text{C}$  to be in exponential growth phase for the ecotoxicity testing. Third, 1 mL of solution 1, 100  $\mu\text{L}$  of solution 2, 100  $\mu\text{L}$  of solution 3 and

100  $\mu\text{L}$  of solution 4 were mixed in a test flask, and FPW was added to bring the final volume to 100 mL. A corresponding control was prepared with deionized water instead of FPW. Finally, clonal algal cells were inoculated into the test and control flasks at an initial density of  $10^4 \text{ cells}\cdot\text{L}^{-1}$  and placed in an illuminated incubator at  $20 \pm 2^\circ\text{C}$ . The optical density of the culture medium in the test and control flasks was measured at 0 h, 24 h, 48 h, 72 h and 96 h. The aquatic ecotoxicity of FPW was quantified based on the difference between algal cell densities in the test and control flasks during the 96-h incubation. There were three replicates ( $n=3$ ) for all samples.

### 2.5 Selection of different microorganisms and plants on FPW

Four types of microorganism mixtures were initially tested separately to identify the best performing treatment based on changes in water quality and aquatic ecotoxicity before and after additions of the mixtures to FPW. Conventional AS was selected for testing because this mixture of microorganisms is widely applied to municipal sewage and industrial wastewater. The three other types of tested microorganism mixtures were commercial microbial preparations designed for water treatment. These mixtures contained multiple advantageous microbes for reducing a variety of different pollutants. The descriptions and concentrations of the four tested microorganism preparations are shown in Table 1. After the best-performing microorganism mixture was selected, it was combined with ten types of plants (ryegrass, water dropwort, typha, reed, iris, canna, water caltrop, rape, water spinach, and *Alternanthera philoxeroides*) to treat the three types of FPWs. All the treatment tests were conducted with 1 L FPW in a 5 L glass container irradiated with a 60 W red lamp and two 40 W blue lamps for 12 days. Detailed information of the candidate plants is shown in Table 2. Every treatment had three replicates.

### 2.6 Determination of microbial diversity and composition in FPW

Variations in microbial diversity and composition in FPW (PW1) were investigated in the treatments of microorganisms and plants, with the aim to improve understanding of the remediation effects and mechanisms of the treatments. Microbial composition and diversity (Shannon-Wiener index) were quantified based on microbial community analyses performed using a high throughput sequencing method [32, 33] at the Chengdu Institute of Biology, Chinese Academy of Sciences. For these measurements, DNA of microorganisms in FPW was first extracted using the MO BIO Power Soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA). The universal primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R (5'-CCCCGYCAATTCMTTTRAGT-3') with a 12-nt unique barcode were used with

PCR to amplify the V4 hypervariable region of 16S rRNA for pyrosequencing with an Illumina MiSeq sequencer [32, 33]. Two PCR amplifications were conducted for each sample and the products were combined. PCR products were subjected to electrophoresis using 1.0% agarose gel. The appropriate band was excised and purified using a SanPrep DNA Gel Extraction Kit (Sangon Biotech, China, Cat# SK8132) and quantified with a NanoDrop instrument. The purified amplification samples were then analysed with an Illumina MiSeq system for sequencing with the Reagent Kit v22×250 bp. The obtained microbial sequence data were then processed using Pipeline–Version 1.7.0 (<http://qiime.org/>). Microbial diversity and composition were determined according to relative abundance of different microbes on the basis of the sequence data.

### *2.7 Data Analysis*

SPSS 16.0 software was used for the statistical analysis. Differences between the treatments were analysed with a one-way analysis of variance (ANOVA) and Tukey's test. Water quality measurements were compared among the treatments. A significance level of  $p < 0.05$  was adopted for all comparisons. A principal coordinate analysis (PCoA) was conducted on microbial species and amount in the different treatments to compare the microbial community diversity and composition differences using Canoco5.

## **3. Results and Discussion**

### *3.1 Selection of microorganisms*

FW (flowback water) and PW (produced water) showed significant differences in the tested water quality parameters (Figure 1). The TN, NH<sub>4</sub>-N, and NO<sub>2</sub>-N in PW were lower than in FW, while NO<sub>3</sub>-N, phosphate and COD<sub>Cr</sub> was higher in PW than in FW. However, there were no significant differences in TP and BOD<sub>5</sub> between FW and PW, which were 1.16~1.47 and 12.4~18.1 mg/L, respectively. In both FW and PW, before wastewater treatment, COD<sub>Cr</sub>, NH<sub>4</sub>-N and TP exceeded by 28.8~35.9-, 1.2~13.2- and 1.2~1.5-fold, respectively, the effluent levels for the petroleum refining industry developed by the Standardization Administration of the People's Republic of China [34]. TN in FW was 4.7 times the effluent standard defined by the industrial wastewater discharge standard of China [34].

All the four mixtures of microorganisms were effective in treating nitrogen-containing (TN) pollutants in FPW. However, the treatment efficiency for removal of TN was lower in PW than in FW, which might result from biological nitrification and denitrification. A high



degree of nitrification occurred in the FW treatments (Figure 1), as indicated by the decreases in  $\text{NH}_4\text{-N}$  and  $\text{NO}_2\text{-N}$  and the increases of  $\text{NO}_3\text{-N}$  [35-37], while in the PW treatments, there were no significant changes in  $\text{NH}_4\text{-N}$  and  $\text{NO}_2\text{-N}$  increased. Meanwhile, high levels of microbial denitrification occurred in the treated FW as reflected by decreases in TN and  $\text{NO}_2\text{-N}$  [38, 39]. A significant reduction in both  $\text{COD}_{\text{Cr}}$  and  $\text{BOD}_5$  was found in all three types of FPWs which indicated favourable processing of organic contaminants. MR-N and MP-R were more effective in reducing  $\text{COD}_{\text{Cr}}$  in FPW, whereas AS was more effective in reducing  $\text{BOD}_5$ , especially in FW. However, with AS, TP reductions in FPW were minimal, and some samples under these treatments showed slight increases in TP.

Aquatic toxicity of the three treated FPW was also evaluated according to the growth reduction and reproduction impairment of the green algae, *Scenedesmus obliquus*. The three FPW were highly toxic for the green algae before treatment by microorganisms (Figure 2). Algal growth and reproduction were almost completely inhibited in the untreated FPW samples. Inhibition of algal population was 83.3~88.7%, 96.3~100%, 98.7~100% and 98.2~100% after incubation in FPW for 24, 48, 72 and 96 h, respectively. Significant differences in the ability to reduce the aquatic toxicity of FPW were observed among the four microorganism mixtures (Figure 3). Untreated FPW showed inhibition of 86.3~87.5%, 92.6~93.5%, and 97.1~97.5% of algae growth and reproduction after 48, 72 and 96 h, respectively. The microbial preparations, MP-N, MP-P and MP-R, provided minimal reductions in the aquatic ecotoxicity of FPW. The inhibition of algal growth after 96 h treatment with the microbial mixtures remained high, at 94.4~98.3%, 96.4~99.0% and 93.5~97.2% for MP-N, MP-P and MP-R, respectively. However, aquatic ecotoxicity of the three FPW was significantly decreased after treatment with AS for 12 days as indicated by significant increases in algal density. Algal cell numbers increased rapidly and growth and reproduction inhibition at 48 h, 72 h, and 96 h was respectively, 52.0~67.7%, 63.6~78.0%, and 72.7~85.1%, which was significantly lower than untreated FPW. Thus, AS was more effective in reducing aquatic ecotoxicity and other environmental impacts of FPW.

These results showed that despite high TDS, organic matter and salt, AS was effective in reducing 34.1~46.5% of COD and 15.3~57.7% of TN from FPW. Conventional AS is an attractive treatment that is widely applied to municipal sewage and industrial wastewater as well as to some high-salinity wastewater, presenting advantages in deionization, sorption and biodegradation and high effectiveness in removing salt and organic carbon compounds [7, 20, 40].

### 3.2 Screening of plants for improved treatment efficiency of AS

Ten candidate plants were chosen to combine with AS for further treatment of FPW. A separate sample collection of FPW was used as a control group (untreated) which was placed under the same conditions as the treated samples for 12 days. Few changes of the water quality parameters were observed for the FPW controls during the 12-day period (Figure 4, control day 1 and control day 12). The results showed different treatment effectiveness for the ten types of plants.

The combination of plants WD, RP, TP, and CA with AS significantly reduced TN, NH<sub>4</sub>-N and NO<sub>3</sub>-N in the FPW. The average concentrations of NO<sub>2</sub>-N showed 1.86~8.24-, 1.00~4.87-, and 0.60~1.20-fold increases in PW1, PW2 and FW, respectively, which indicated NO<sub>2</sub>-N was produced from TN and NH<sub>4</sub>-N due to incomplete microbial nitrification and denitrification [35-37]. A higher level of microbial transformation might be required to eliminate these nitrogen-containing contaminants thoroughly. Compared with the treatment of AS alone, the combination of the plants RD, WD, WC, TP, WS, AP, and IS with AS significantly improved TP and phosphate with reductions in FW of 68.4~74.8% and 7.7~43.1%, respectively, while a minimal effect was observed in PW. The combination of AS with most of the studied plants exhibited better performance in reducing COD<sub>Cr</sub> and BOD<sub>5</sub> in FPW.

The changes of algal density with exposure to FPW treated with AS and plants are shown in Figure 5. Among the studied plants, WD combined with AS appeared to be the best option with the most effective treatment efficiency and favourable algal growth. The effectiveness of the treatments combined with AS was in the order of WD > RG > WC > AP for PW1 and WD > IS > WC > WS for PW2. In FW, the effectiveness of the combined plant and AS treatments were as follows: WD > WS > IS > WC. The reduced algal toxicity effects were in agreement with the improvements in several water quality parameters, which showed (Figure 4) decreases of 35.2~78.0%, 17.3~99.1%, 22.6~76.4%, 16.8~51.4%, and 63.5~98.4% for TN, NH<sub>4</sub>-N, NO<sub>3</sub>-N, COD<sub>Cr</sub> and BOD<sub>5</sub>, respectively.

Synergistic effects between plants and microorganisms were also found for the improvement of FPW water quality and reduction of algal toxicity. Complex processes in removing organic and inorganic pollutants were induced by plant and microorganism synergisms in the wastewater treatments [41, 42]. In particular, the microorganisms in AS were capable of decomposing organic material and reducing the toxicity of hazardous pollutants in the wastewater treatment process [43, 44]. The reduced toxicity of FPW improved plant health and growth. At the same time, the plants could provide a favourable environment with dense and extensive roots and abundant nutrients for the growth and

reproduction of microorganism and thereby enhance the remediation efficiency of AS [37, 45-47]. The plants could also provide oxygen for microbial aerobic degradation of organic pollutants, which would contribute to the better survival and performance of AS [23, 24]. In addition to the synergistic partnerships with AS, WD (water dropwort), IS (iris), CA (canna), and AP (*Alternanthera philoxeroides*) were also found to be effective plant species for water treatment with higher uptake and removal efficiencies of extensive pollutants including TN, NH<sub>4</sub>-N, NO<sub>3</sub>-N, NO<sub>2</sub>-N, TP, COD, phosphorus, and microcystins [46, 48-51]. Our results showed that the combination of the plant WD with AS was the most effective treatment for reducing COD (39.5~51.4% reduction), TN (62.9~78.0% reduction) and TP (4.4~96.5% reduction) in FPW.

### 3.3 Variations in microbial community diversity and structure of FPW

Microbial community diversity (Shannon-Wiener Index) in PW1 was examined before and after the combined treatments of plants and microorganisms to evaluate the treatment effects (Figure 6). The Shannon-Wiener index, which is commonly used to characterize both the abundance and evenness of the species present in a community. As shown in Figure 6, the Shannon-Wiener index in FPW increased from day 0 (FPW0) to day 12 (FPW12) in the control, without plant or microorganism additions. However, the combined treatments of plants and AS showed improved Shannon-Wiener indices (from 6.32 to 7.98) compared with FPW12 indicating the effectiveness of the treatments with plants and AS. Among the ten plants, WD had the greatest effect on improvement of microbial community diversity (with a mean value of 7.98), which was consistent with previous results of aquatic ecotoxicity reduction and water quality improvement.

A principal coordinate analysis was conducted of the microbial community in PW1 under different treatments. PCoA results showed that the microbial community composition of PW in all the treatments was obviously different than that of the untreated PW (point FPW0) and AS (point AS0) (Figure 7), which demonstrated that the variety and number of microbes in the treated PW noticeably changed. Additionally, the microbes *bacteroidetes*, *verrucomicribia*, *tenericutes*, and *spirochaetes* were the most relevant variables associated with all the WD, WC, WS and AP treatments, and showed better performance in decreasing the algal toxicity of FPW. It might also demonstrate that these microbial species were directly related to the treatment efficiency. Alterations in taxonomic composition and relative abundance of microbes in each treatment is shown in Figure 8. The untreated PW was lower in microbial varieties (Fig. 8, group PW0), and *proteobacteria*, *euryarchaeota* and *synergistetes* were the dominant microbe species (Fig. 7 and Fig. 8), which was consistent with previous reports [52].

However, the relative abundance of anaerobic or aerobic microbes *Bacteroidetes*, *firmicutes*, *chloroflexi*, *actinobacteria*, *spirochaetes*, *planctomycetes*, and *verrucomicribia* was greatly stimulated in the combined treatment of the plant WD and AS, while that of *proteobacteria*, *euryarchaeota* and *synergistetes* was significantly decreased. Several of the stimulated microbes are specialized in their adaptive strategies for surviving extreme conditions and might have different mechanisms for the removal of pollutants. For example, *bacteroidetes*, *firmicutes* and *chloroflexi* can survive in extreme conditions by strategies such as producing endospores, using oxygen and growing well in high temperatures, using toxic halogenated organics as electron acceptors, using light for photosynthesis and producing energy through photosynthesis [53, 54]. *Planctomycetes* has a unique cell wall and large cell membrane invaginations which may be related to its biosynthesis abilities, enabling it to take in large molecules via a process used by some eukaryotic cells to engulf external items [55, 56]. Additionally, *Actinobacteria* could have a synergistic effect with plants while living symbiotically with them by fixing nitrogen for uptake by plant roots in exchange for access to saccharides released from the plant. The *Actinobacteria* further acts as fungi to decompose organic matter, enabling pollutant molecules to be taken up anew by the plants [57]. Perhaps the microbial preparations can first survive exposure to FPW and then have remediation effects as a result of their high decomposition and degradation abilities for pollutants. In contrast, *proteobacteria*, *euryarchaeota* and *synergistetes* are previously identified as significant contributors to the fixation and degradation of contaminants due to their diverse metabolic properties and wide variety in metabolism types [58-60]. However, the amount of these microbes decreased in the treatments of the present study, which were quite different than in wastewater treatment plants, probably due to the distinct characteristics of FPW.

AS (Fig. 8, group AS0) exhibited high microbial biodiversity and was abundant in various microbes such as *proteobacteria*, *bacteroidetes*, *chloroflexi*, *actinobacteria*, *planctomycetes*, etc., which can be seen in the PCoA results. However, few effects were found in the treatment of PW (Fig. 8, group AS), which indicated that treatment efficiency might not only depend on microbial biodiversity but also on community composition.

#### 4 Conclusions

- (1) Activated sludge was effective in reducing COD, TN and aquatic ecotoxicity of FPW and was more effective than the three other microorganism preparations.
- (2) Water dropwort provided the best synergistic partnership with activated sludge, and of the ten aquatic plants that were combined with activated sludge, water dropwort had the

- highest treatment efficiency (improved water quality and reduced aquatic ecotoxicity).
- (3) Microbial abundance and diversity of the treated FPW were greatly increased as reflected by the Shannon-Winner index and the microbial community composition.
- (4) This study implies that phyto-micro remediation has good potential for treating FPW from unconventional shale gas exploration considering both its low cost and easy deployment.

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## **6 Compliance with Ethical Standards**

The authors declare that we have no conflicts of interest. This manuscript is approved by all the authors, and it has not been submitted to more than one journal for simultaneous consideration. The manuscript content has not been published before. The submitted work has not received any financial support from a third party, and there is no financial relationship with any of the entities. All of the financial organizations associated with this work have been disclosed. There is no patent, whether planned, pending or issued, that is broadly relevant to the submitted work.

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**Table 1**

The concentrations (mg/L) of three microbial preparations and the MLSS concentration (mg/L) of activated sludge for three FPW samples

NO.	Name	Abbreviations	Concentration	Description
1	Activated sludge	AS	407.2±4.5	—
2	Microorganism N	MP-N	406.3±1.7	Composed by a high quantity of active colonies of broad spectrum microbes for reducing nutrients in natural water bodies
3	Microorganism P	MP-P	406.3±0.7	Containing beneficial spore forming bacillus, enzymes, yeast and fungi for reducing heavy metals, toxic ammonia and unwanted excess phosphorus in water bodies
4	Microorganism R	MP-R	407.8±1.9	Consisting of special beneficial microbes with natural plant enzymes for reducing organic sediment in water bodies

**Table 2**

The biomass (g) and abbreviation of the candidate plants for the three FPWs

NO.	candidate plants	Abbreviations	Biomass (g)	Root volume (mL)
1	Ryegrass	RG	138.70±0.99	50.7±1.5
2	Water Dropwort	WD	105.27±7.52	49.8±1.3
3	Typha	TP	85.10±5.61	50.9±1.6
4	Reed	RD	55.90±6.16	48.7±2.4
5	Iris	IS	97.09±9.85	49.4±2.2
6	Canna	CA	42.37±7.51	50.2±1.7
7	Water Caltrop	WC	30.57±0.47	49.6±1.8
8	Rape	RP	66.71±3.50	50.2±1.4
9	Water Spinach	WS	43.63±2.12	49.9±1.5
10	<i>Alternanthera philoxeroides</i>	AP	37.26±1.12	50.8±1.1

## Figure legends

**Fig. 1.** The water quality changes in three FPW samples treated with different microorganism mixtures

**Fig. 2.** Aquatic ecotoxicity of three FPWs without microorganism and plant treatments

**Fig. 3.** Algal ecotoxicity of three FPWs after treatment with different microorganism mixtures

**Fig. 4.** The water quality changes in three FPW samples treated with different plants combined with activated sludge

**Fig. 5.** Algal ecotoxicity of three FPW samples after treatment with different plants combined with activated sludge

**Fig. 6.** Variations in microbial community diversity in produced water under different treatments (PFW0: untreated PFW; PFW12: PFW after 12 days without any treatments)

**Fig. 7.** Principal coordinate analysis (PCoA) of microbial communities in produced water under different treatments (PFW0: initial PFW; PFW12: PFW after 12 days without any treatments; AS0: before treatment with AS)

**Fig. 8.** Relative abundances of microbial community in produced water under different treatments (PFW0: untreated PFW; PFW12: PFW after 12 days without any treatments; AS0: before treatment with AS)

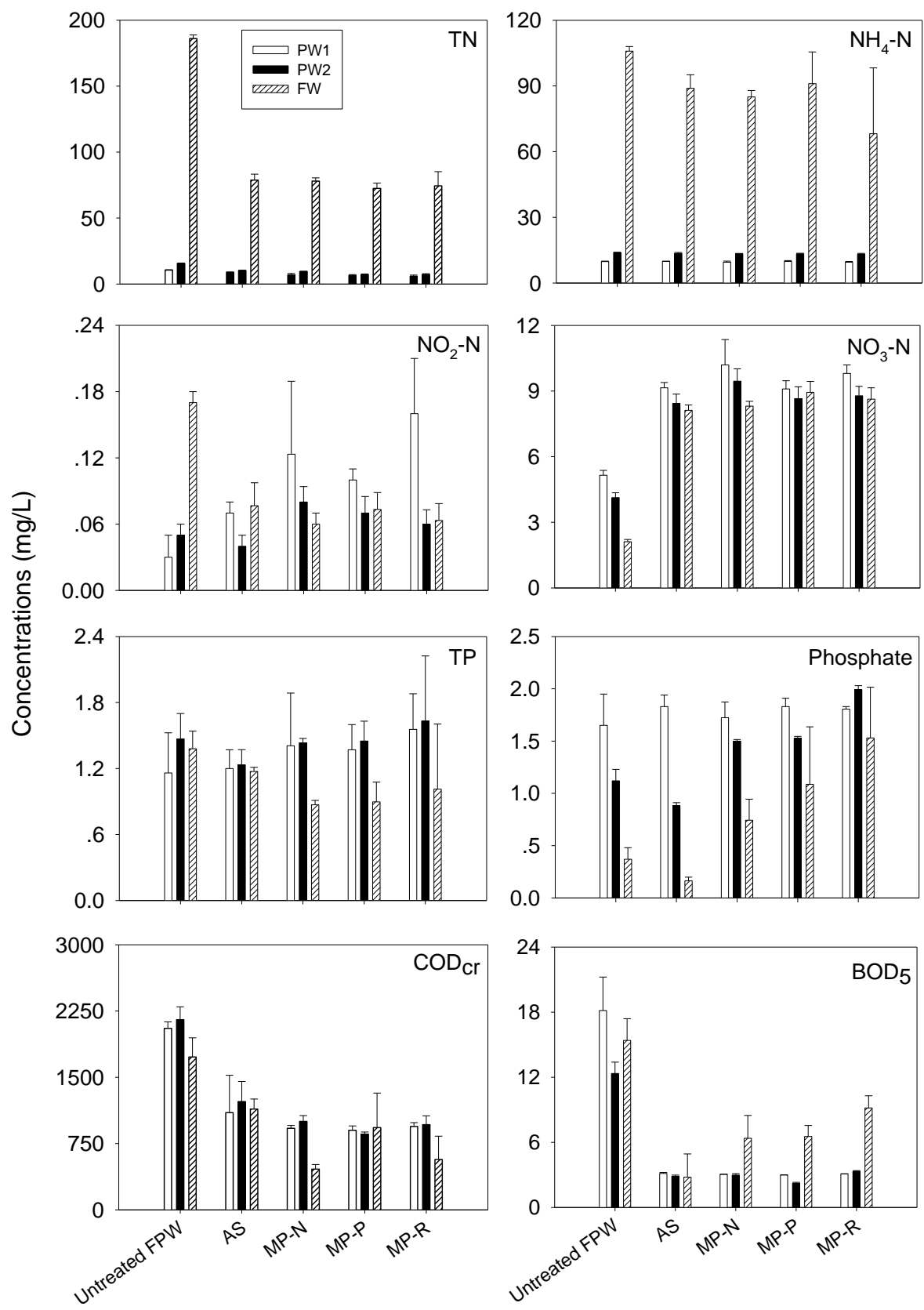


Fig. 1

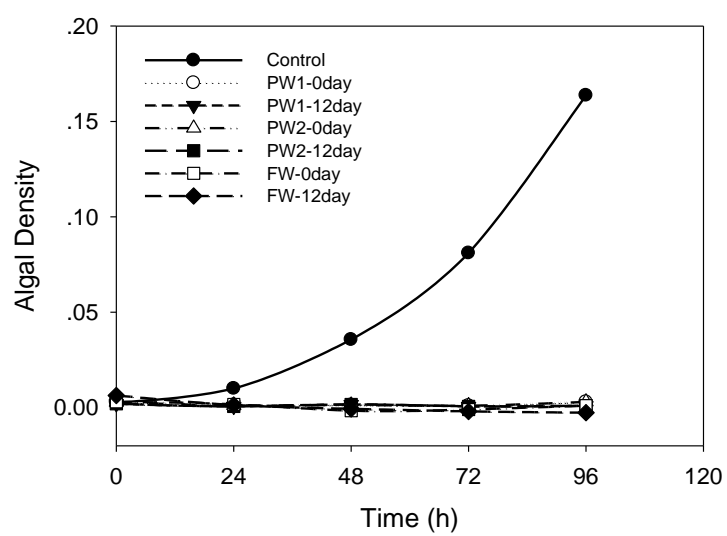


Fig. 2

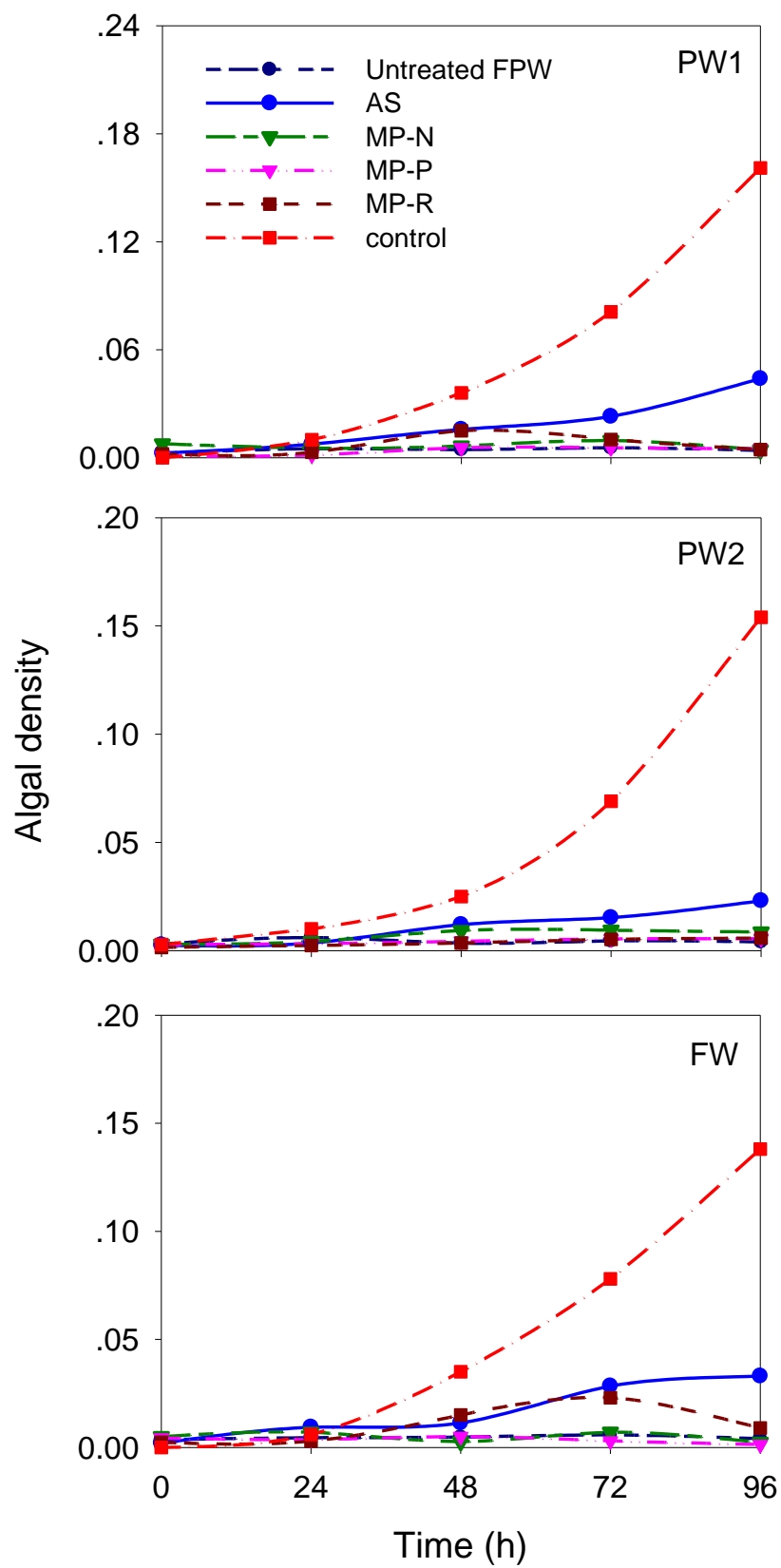


Fig. 3

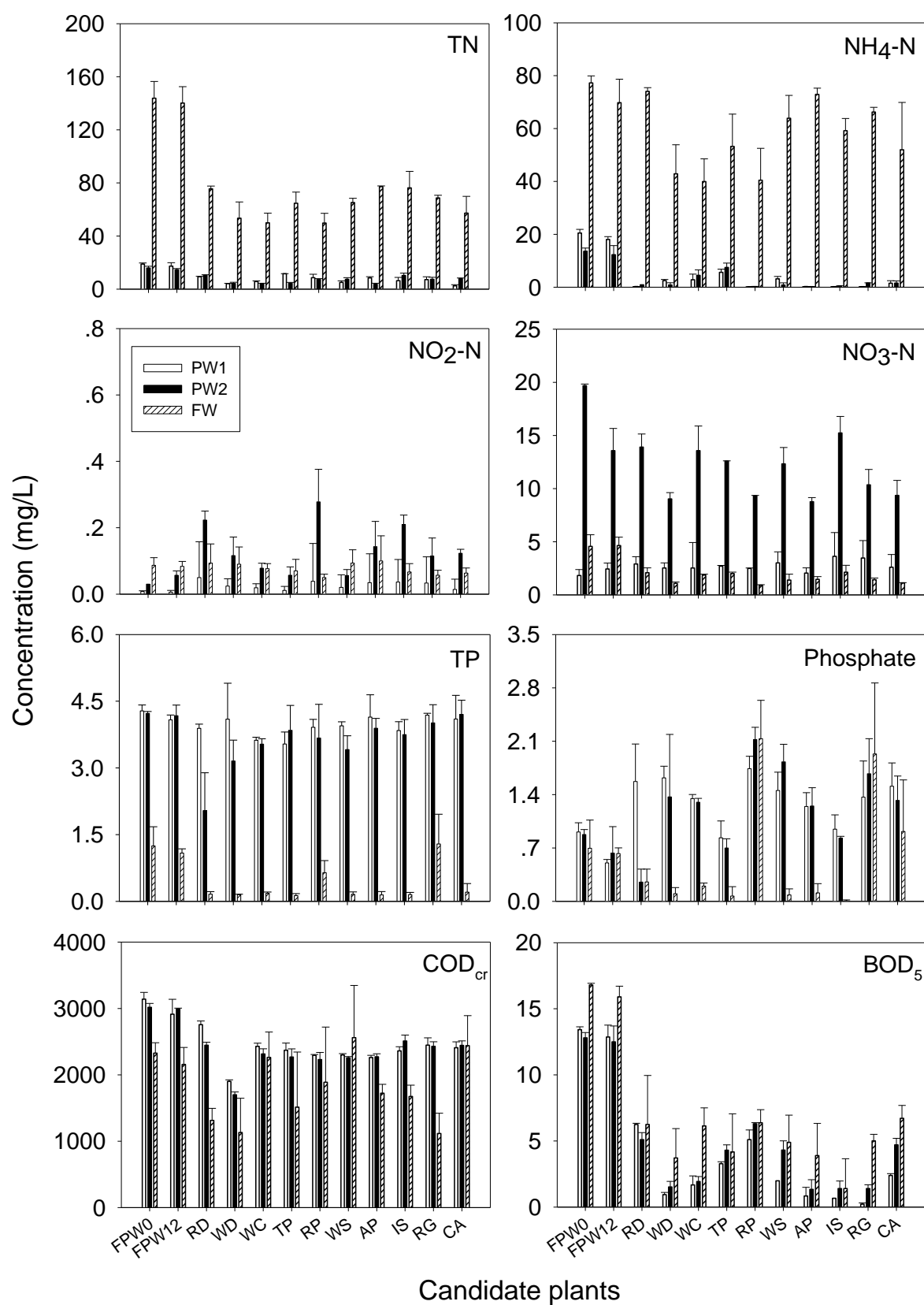


Fig. 4

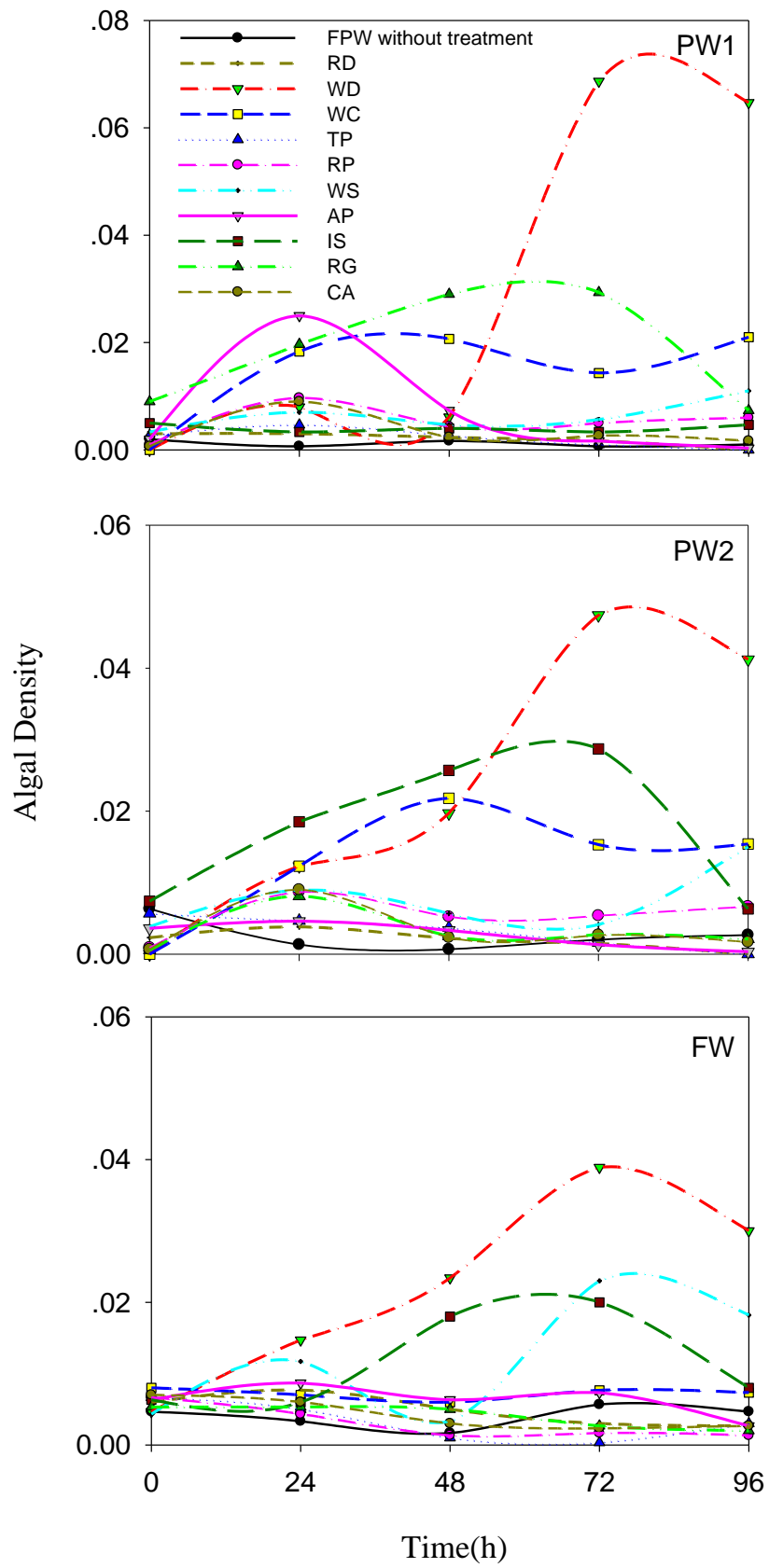


Fig. 5



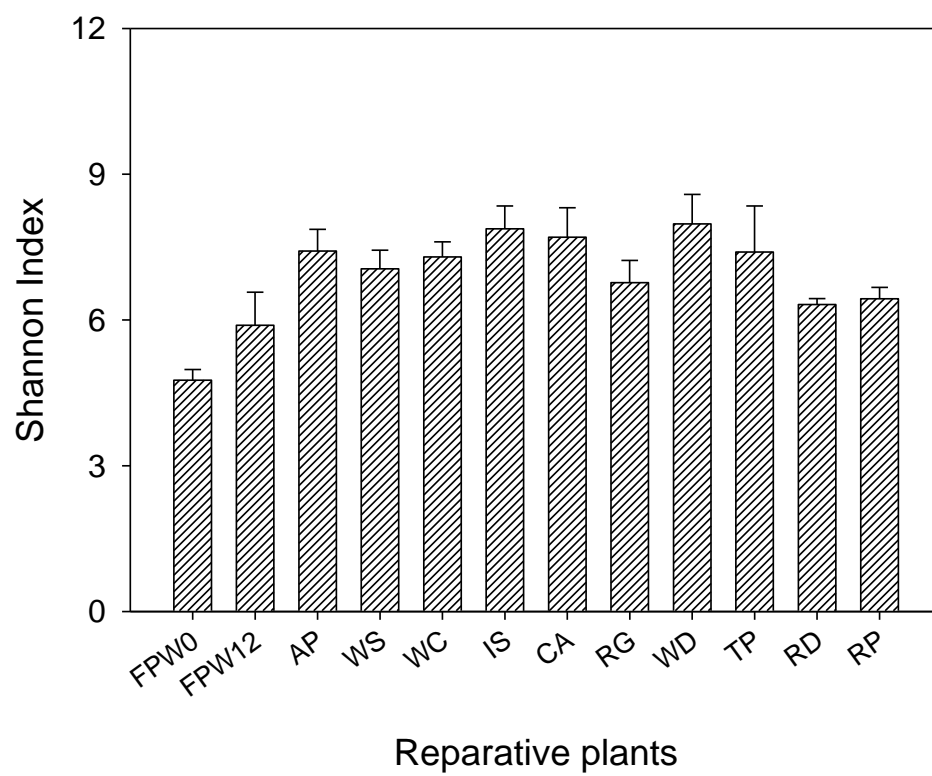


Fig.6

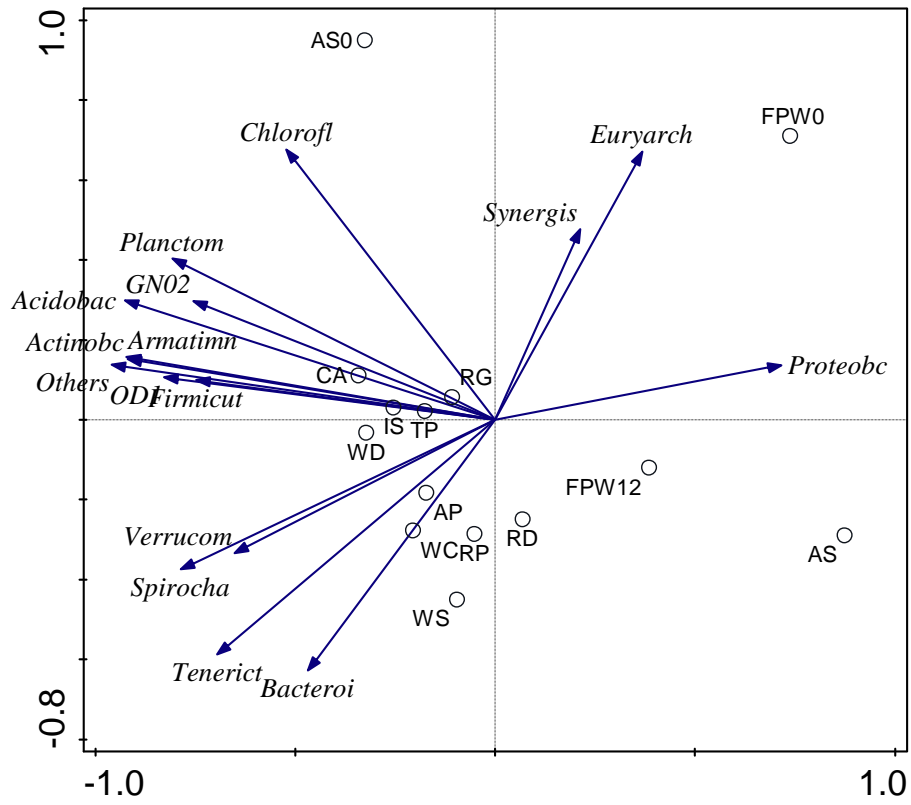


Fig.7

